Monitoring Metabolite Dynamics in Patient-Derived Tumoroids using Automated Microfluidic-based Pu·MA System

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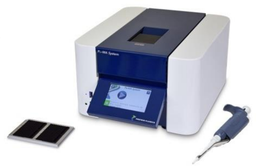
INTRODUCTION

Cancer cells are characterized by metabolic modifications to meet high bioenergetic and anabolic demand during tumorigenesis. For most tumors, aerobic glycolysis is a major driver of cancer progression, resistance to chemotherapy, and poor patient outcome. Lactate, a product of glycolysis, plays a role in tumor progression and is linked to drug resistance in breast cancer. Patient-derived 3D cancer cell models are valuable tools for research, drug development and personalized medicine, because they recapitulate features of tumor microenvironment. Therefore, monitoring metabolite dynamics and lactate production in physiologically relevant patient-derived models can provide valuable data for understanding metabolic perturbations during disease progression, drug response and resistance. In this study we assayed the glucometabolic changes in response to anti-cancer drugs in triple negative breast cancer (TNBC) tumoroids. Our approach utilizes an automated 3D assay system. The Pu·MA system with microfluidic flowchips allows for *in situ* supernatant sampling, sensitive luminescence Lactate-Glo assay and high content imaging.

INSTRUMENTATION

The Pu·MA System and flowchips

- Automated media exchanges occur with cells in protected chamber
- Supernatants can be collected to monitor cell secretion
- Cells can be lysed in situ for sensitive metabolomic profiling
- Spheroids can be imaged in the flowchip, or samples removed for immunoassay or metabolomics analysis



ImageXpress Micro Confocal High-Content Imaging System

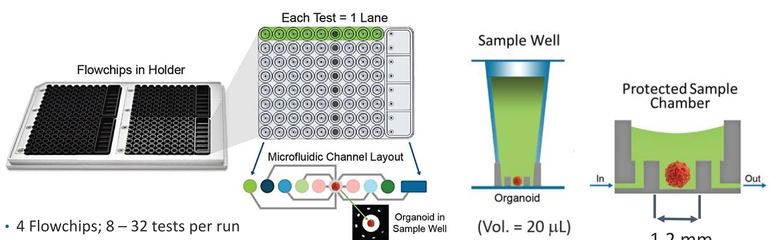
- Five colors + transmitted light
- Environmental control
- Automated Data analysis
- The system is controlled by MetaXpress High-Content Image Acquisition and Analysis Software

GloMax®-Multi+ Detection System with Instinct® Software

- Expandable multimode reader with dedicated optics for each mode
- Luminescence, Fluorescence, UV-Visible Absorbance

Pu·MA MICROFLUIDICS FLOWCHIP

Each Pu·MA System flowchip contains eight lanes of reagent wells connected by microfluidic channels. Four flowchips are placed in holder that locates all wells in a 384 multiwell plate format providing for 32 samples per assay. Each test lane designated to one organoid or spheroid sample and consists of sample well connected to the reagent wells via microfluidic channels. Flowchip can be filled with any reagents (media, compounds, stains, etc.) depending on the assay configuration. Organoids/spheroids are loaded into the sample well and located in a protected chamber at the bottom of the well. This allows reagents to be directed in and out of the sample well without disturbing or drying out the microtissue. The bottom of the flowchip is a thin cyclic olefin copolymer (COC) film which makes it compatible with high resolution imaging.



- 4 Flowchips; 8 – 32 tests per run
- Wells are on 384-plate spacings
- Optically clear bottom compatible with imaging

Figure 1. Schematic of flowchips showing channel layout and sample well with proprietary protected chamber. The diameter of the sample well clear aperture is 1.2 mm.

References
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PATIENT-DERIVED TUMOROID FORMATION

Model System
- TU-BcX-4IC cells were derived from a primary tumor
- Classified as Metaplastic breast cancer (MBC) with a TNBC subtype
- Tumor exhibited resistance to therapy with adriamycin, cyclophosphamide and paclitaxel

Approach
- Tumoroids were formed from cultured TU-BcX-4IC cells (as shown in **Fig 2**)
- 2K – 4K cells per well in 96 or 384-well ultra-low attachment plate
- Treatment up to 72 hours in flowchips

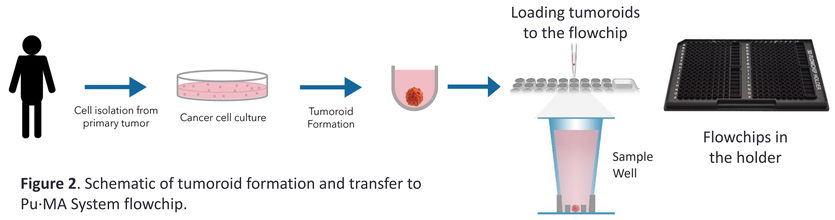


Figure 2. Schematic of tumoroid formation and transfer to Pu·MA System flowchip.

METABOLITE SECRETION ASSAY

4IC tumoroids were treated overnight with compounds. Media + Compound was exchanged every 3 hours to provide a time-course sampling of metabolite secretions. Supernatants were analyzed for lactate using the Lactate-Glo assay kit. Supernatants were diluted 1:20 in media. (**Fig 3**)
10 µL Samples + 10 µL assay reagents added to 384 multiwell plates. Plates were incubated for 60 minutes then luminescence read on GloMax plate reader

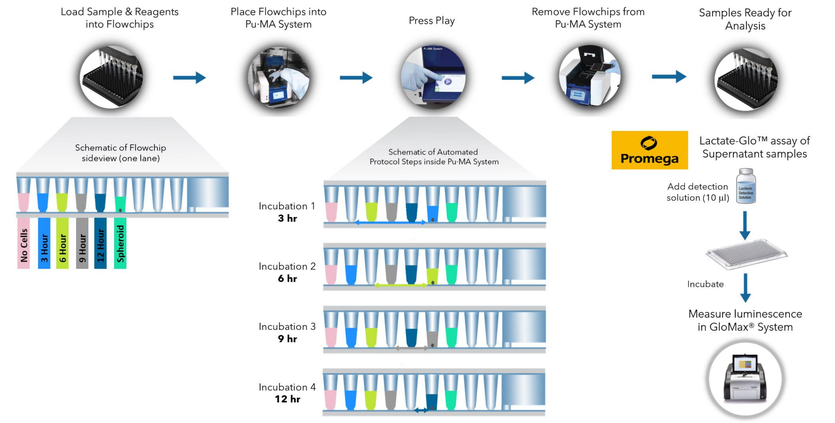


Figure 3. Workflow of metabolite secretion assays. Supernatants are sampled in situ by Pu·MA System, collected in the flowchips, then transferred into 384 well plates where they were analyzed for Lactate-Glo assay in a GloMax luminescence plate reader (Promega)

TUMOROID VIABILITY ASSAY

To assess tumoroid uniformity and viability before drug treatment, we optimized the RealTime-Glo® assay for 3D cell-based models (from **Promega, Fig 4A**). The reagents were transferred to the tumoroid samples within the flowchips, and luminescence was measured using GloMax plate reader. Assay demonstrated good linearity of the luminescence signal over a range of tumoroid cell density (**Fig 4B**). Coefficient of variation of signal from samples = 26% indicates high starting uniformity of tumoroid size & viability (**Fig 4C**), which can be used as data normalization factor.

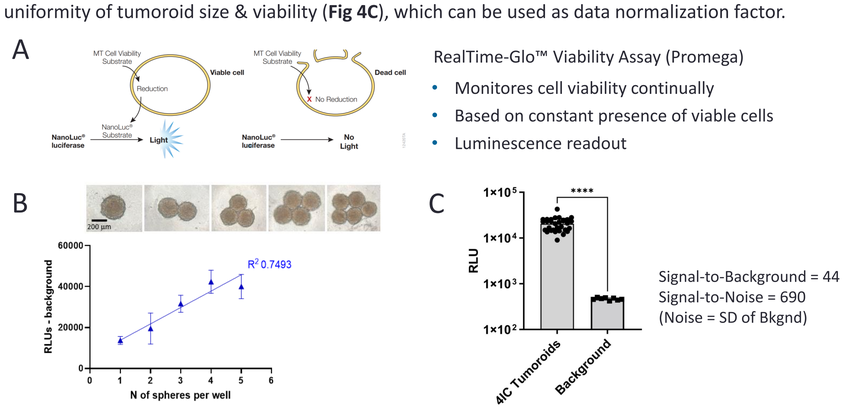


Figure 4. **A.** Schematic of RealTime-Glo assay (Promega). **B** and **C** Tumoroid viability and size uniformity measured by luminescence using RealTime-Glo assay within flowchips.

TUMOROID LACTATE-GLO ASSAY

4IC tumoroids were treated overnight with compounds:
- Romidepsin – A histone deacetylase (HDAC) inhibitor
- Trametinib – Selective reversible allosteric inhibitor of MEK1 and MEK2 activity
- Paclitaxel – A mitotic inhibitor that interferes with microtubule growth

Tumoroid treatment of over 9 – 12 hr also resulted in statistically significant increase in lactate secretion for paclitaxel ($p < 0.05$) and romidepsin ($p < 0.01$) indicating drug-induced shift toward glycolysis.(**Fig. 5**) These results corroborate previous publications linking treatment with paclitaxel to increased lactate production and chemoresistance in breast cancer.

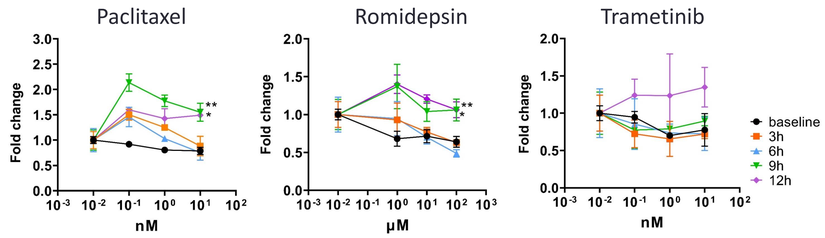
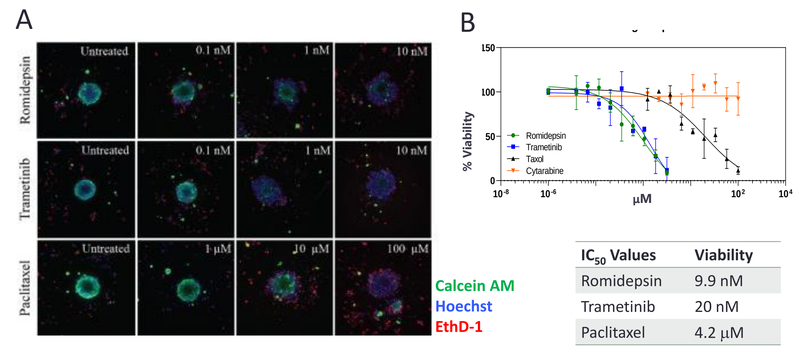


Figure 5. Relative amount of lactate measured in tumoroid supernatant samples as a function of compound concentration and incubation time. Data is shown as fold change relative to the baseline measurement (3 hr incubation with no compound). Each data point represents 3 independent tumoroids. Error bars = +/- 1 SDM

TUMOROID RESPONSE TO TREATMENT

Treatment of 4IC tumoroids for 48 hr with paclitaxel, romidepsin and trametinib showed varying sensitivities to these drugs. IC_{50} values for romidepsin and trametinib were ~10 nM, as compared to paclitaxel >1 mM, which is much greater than typically reported for breast cancer models. (**Fig 6**). This is consistent with previously published taxane resistance of 4IC primary tumor. [1,2]



IC_{50} Values	Viability
Romidepsin	9.9 nM
Trametinib	20 nM
Paclitaxel	4.2 µM

Figure 6. Drug response of 4IC tumoroids treated with different concentrations of paclitaxel, romidepsin and trametinib. **A** Tumoroids were stained with Hoechst, Calcein AM and EthD-1 and with 10X objective. Presented are maximum intensity projection images. **B** Image analysis was done of in 3D with Z-stack of images using Custom Module Editor. Data was translated into dose response curves with reported IC_{50} values.

CONCLUSIONS

- Pu·MA System performs automated complex organoid assay protocols with 3D cell models within the incubator environment.
- Samples are protected from damage or loss during assay in Pu·MA System.
- Metabolite dynamic automated workflow in patient-derived tumoroids is a valuable tool for studying disease progression, drug resistance, identifying new therapies and advancing personalized medicine.
- Further work is on-going to determine relation of metabolism dynamics to compound mechanisms of action.

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